Intra- and Interpopulation Variability of Thermal Properties of Maize Starch[1]

PAMELA WHITE,[2] IBRAHIM ABBAS,[3] LINDA POLLAK,[4] and LAWRENCE JOHNSON[5]

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ABSTRACT

The variability in thermal properties of laboratory-isolated starch from five open-pollinated populations of genetically variable maize was evaluated using differential scanning calorimetry (DSC). Each population was represented by ears from four self-pollinated plants, and starch from five kernels of each ear was individually extracted and analyzed. The data among populations showed significant differences ($P < 0.05$) in the DSC values of the isolated starch, suggesting differences in composition and degree of crystallinity of the starches. The DSC values measured include peak onset, temperature range of gelatinization, and enthalpy. Further investigation was made to evaluate the variability in thermal properties of the starch among maize plants of the same population by using ears from 20 self-pollinated plants (five kernels each) of one population. Results showed significant differences among plants within the same population, indicating that genetic variability for starch structure and, thus, thermal behavior of the starch, may exist within populations.

Stevens and Elton (1971) first described the use of differential scanning calorimetry (DSC) for the study of starch gelatinization. Since then, DSC has been used extensively to study the gelatinization of starch, with many reports appearing around 1980 (Biliaderis et al 1980, Ghiasi et al 1982, Donovan et al 1983, Knutson et al 1982). The application of DSC to starch gelatinization has considerably improved the understanding of this property (Hoseney 1984).

Throughout these studies, researchers have attempted to relate certain DSC characteristics to starch composition or characteristics. For example, Stevens and Elton (1971) noted that waxy (*wx*) cornstarch produced a wider gelatinization peak than normal cornstarch and that the endotherm for high-amylose cornstarch began at the same temperature as that for normal corn, but it had no clear peak and was not not complete until 115°C. Biliaderis et al (1980) reported that branching is detrimental to crystallization, that it lowers the true melting point of undiluted synthetic polymer and widens the temperature range of melting. In contrast, Krueger et al (1987b) reported that starches from normal and mutant corn varieties with the highest amylopectin content have the narrowest temperature range of gelatinization.

Knutson et al (1982) found that gelatinization temperature, range, and enthalpy (ΔH) of amylomaize increase with decreasing granule size. They suggested that, when granules are small, there are more particles per sample, resulting in more heterogeneous granules, which, in turn, broadens the enthalpy range. Additionally, they speculated that gelatinization behavior is influenced by the permeability of the surface and the internal pressure within the granule required to disrupt that surface.

In studying DSC properties of maize, most researchers have examined only hybrid dent corn or maize containing the major endosperm genes (i.e., waxy [*wx*] and amylose-extender [*ae*]) (Brockett et al 1988). However, the DSC properties of starch from several maize genotypes (normal, *ae*, *wx*, dull [*du*], and sugary2 [*su2*]) were evaluated by Inouchi et al (1984) at different stages of growth. It was shown that the ΔH values declined in the order of *wx*, normal, *du*, and *su2* starches. Brockett et al (1988) found similar results, with ΔH values declining in the order of *ae wx*, *wx*, *ae*, normal, and *du* endosperm of sweet corn inbred Ia5125. Krueger et al (1987a) examined the effect of inbred line differences on the thermal properties of normal maize starch. They found that starches from different maize varieties had significant variations in thermal properties, especially in ΔH. Peak temperature and temperature range of gelatinization showed less variation. They suggested that it may be possible to identify maize genotypes by comparing their ΔH values and peak height index, a value described as the ratio of ΔH to half the range.

Mutant genes of maize can have substantial effects on kernel and starch granule development and morphology, as well as on the polysaccharide composition (Creech 1968, Yeh et al 1981). Sandstedt et al (1968) indicated that starch properties (amylography, water absorption, and susceptibility of raw starches to amylase action) can vary widely with changes in the genetic composition of maize. Further study of the relationship between genetic variation in maize, starch properties, and gelatinization is highly desirable. However, when comparing DSC data from different experiments, one must be certain that pretreatment of the starches and DSC conditions are alike, because these factors will significantly affect the gelatinization behavior.

The objectives of the current study were to determine the amount of variability in thermal properties of maize starch from 1) different open-pollinated populations and 2) different plants of the same population. The populations are old open-pollinated varieties that are unimproved and genetically variable.

MATERIALS AND METHODS

Maize Populations

Five maize populations (Table I) were used to evaluate differences in the gelatinization properties of starch isolated from single kernels. The first four populations (country of origin USA) were dent maize grown in Ames, IA, in 1983. These populations originally were grown in the southern USA before hybrids were developed. The last population, a photoperiod-sensitive floury population from Paraguay, was grown in a winter nursery in Isabela, Puerto Rico, during 1986–1987. These populations are genetically variable for appearance and yield. We wanted to determine whether there were also variations in the thermal properties.

TABLE I
Maize Populations Studied

Population	Name	Kernel Color	Kernel Type	Origin
83:3001[a]	Weekly	White	Dent	USA
83:3011[a]	Herring	White	Dent	USA
83:3021[a]	Nicholas	Yellow	Dent	USA
83:3031[a]	Horse Tooth	Yellow	Dent	USA
86-87:441[b]	MKT, Asuncion	Yellow	Flour	Paraguay

[a]Source: A. R. Hallauer, Iowa State University.
[b]Source: PI 162929, North Central Regional Plant Introduction Station, Ames, IA.

[1]Journal Paper no. J-13159 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Project no. 2568.
[2]To whom correspondence should be addressed at the Food and Nutrition Department, 111 Mackay Hall, Iowa State University, Ames 50011.
[3]American Maize-Products Co., 1100 Indianapolis Blvd., Hammond, IN 46320-1094.
[4]Department of Agronomy, Iowa State University.
[5]Department of Food Technology, Iowa State University.

Plants were self-pollinated and ears were harvested at full maturity. After harvest, corn ears were dried at 38°C for five days to 13% moisture content. The samples were stored in a cold room at 4°C and 45% relative humidity until the kernels were needed for analyses.

To determine interpopulation variability, four plants from each of the five populations were used. Starch from five kernels of each plant was individually extracted and analyzed. For intrapopulation variability, 20 plants (five kernels each) were evaluated from population 83:3001.

Single-Kernel Starch Isolation

Corn kernels were weighed and placed into 15-ml test tubes. Two milliliters of 0.45% sodium metabisulfite (Schoch 1957) was added to each tube. They were then incubated in a water bath at 50°C for about 48 hr. After incubation, the solution was decanted and discarded. The seed coat was removed, and the kernel was degermed by hand. The endosperm was ground with a mortar and pestle and quantitatively transferred into a microcontainer assembly (Fischer part no. 14-509-28), and 5 ml of distilled water was added. The microcontainer assembly is a small blender container that attaches to a regular blender, allowing for proper blending of small samples of about 10 g or less. The mixture was then blended for 3 min at moderate speed. The slurry was screened through a series of sieves (91, 63, 41, and 30 μm). The residue on the screen was discarded, and the screened fluid was transferred into a 50-ml beaker and allowed to settle for 1.5 hr at 4°C. The water layer was decanted, and the starch resuspended in distilled water and allowed to settle at 4°C. The washing-sedimentation process was continued until a clear water layer was obtained. The water layer was decanted and discarded, and the starch was allowed to dry overnight at ambient temperature in a room with a fan circulating air.

Protein content of combined starch samples was determined periodically by using the Hatch Digesdahl method (Hach et al 1987). Samples (500 mg) were digested in 5 ml of sulfuric acid for 5 min. Then 5 ml of 50% hydrogen peroxide was added, and the mixture was heated an additional 5 min and diluted to 100 ml. Protein content was determined on a 10-ml aliquot. Protein contents ranged from 0.58 to 1.00% and averaged about 0.88%.

Differential Scanning Calorimetry

DSC studies were performed by using a Perkin-Elmer DSC7 equipped with a thermal analysis data station. The instrument was calibrated by using indium and zinc standards. To determine instrument and operator variability, 20 samples of regular maize starch were analyzed. For peak onset, range, and enthalpy, the following values and standard deviations were obtained: 65°C (SD 1.26), 8.9°C (SD 0.37), and 2.61 (SD 0.09), respectively.

Approximately 3.5 mg (dwb) of starch was weighed accurately into an aluminum pan, followed by the addition of 8 mg of distilled water. This ratio of starch to water provided enough water to achieve one symmetrical DSC gelatinization peak, yet was not too dilute to reduce peak sharpness. The pan was hermetically sealed and allowed to sit for at least 2 hr. An aluminum pan with 8 mg of distilled water was used as a reference. Samples were heated from 30 to 120°C at rate of 10°C/min. Enthalpy (ΔH), peak onset (T_o), and peak (T_p) temperatures were computed automatically. The gelatinization range was computed as $2(T_p - T_o)$ as described by Krueger et al (1987a). At the water level used, the endotherms were essentially symmetrical, which allowed the total gelatinization range to be established accurately by doubling the $(T_p - T_o)$ value (Krueger et al 1987a). A typical DSC thermogram is shown in Figure 1.

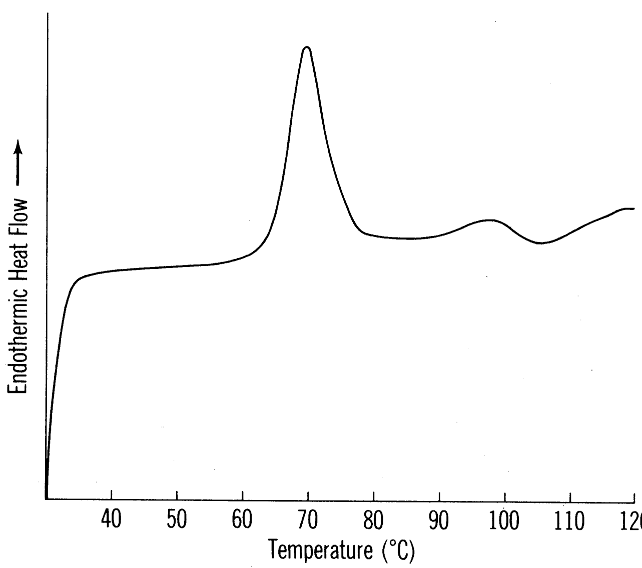

Fig. 1. Typical differential scanning calorimetry thermogram of native maize starch from population 83:3001.

Statistical Analysis

Analysis of variance (Steel and Torrie 1960) was used for data analysis. When F values were significant, mean differences were compared by using least significant differences (LSD) at the 5% level of probability.

RESULTS AND DISCUSSION

Starch gelatinization has recently been described as the collapse (disruption) of molecular orders within the starch granule manifested in irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence, and starch solubilization (Atwell et al 1988). Information on the thermal properties of native starches may help determine variations in their structure or configuration.

TABLE II
Interpopulation Variability in the Differential Scanning Calorimetry Properties of Maize Starch Plant Populations

Population	Plant[a]	T_o(°C)	$2(T_p - T_o)$ (°C)	ΔH (cal/g)
83:3001	1	62.5	15.3	2.39
	2	62.2	15.5	2.13
	3	60.0	19.1	2.36
	4	61.6	15.5	2.51
	Mean	61.6	16.4	2.34
83:3011	1	61.6	16.0	2.56
	7	64.2	9.7	2.82
	8	66.6	9.3	3.05
	26	62.0	11.8	2.71
	Mean	63.6	11.7	2.78
83:3021	1	64.5	11.1	2.22
	2	63.2	13.6	2.36
	7	66.1	10.0	2.71
	27	66.4	9.5	2.85
	Mean	65.1	11.1	2.54
83:3031	5	61.3	17.6	2.49
	10	62.9	12.0	2.47
	15	61.6	13.4	2.36
	20	64.3	13.8	2.77
	Mean	62.5	14.2	2.53
86-87:441	1	67.0	9.0	3.16
	6	67.4	8.9	3.13
	11	67.4	8.0	3.08
	16	66.8	8.7	3.13
	Mean	67.2	8.7	3.13
$LSD_{0.05}$, means		2.21	3.17	0.25
$LSD_{0.05}$, plants within a population		1.40	2.55	0.24

[a] Each plant represents mean data from five kernels.

Interpopulation Variability of Maize

The variability among populations of the thermal properties of starch was evaluated by using five open-pollinated maize varieties. Variations among plants of the same populations also are reported. Results are presented in Table II. Four plants from each maize variety were selected randomly. Five kernels from each plant were analyzed individually, so data listed for each plant represent the mean of five kernels. Least significant differences were calculated to determine differences among the populations as well as among plants within a population.

The DSC mean values of populations indicate statistically significant differences ($P < 0.05$) among populations for T_o, range, and ΔH. The most significant difference was noticed when 86-87:441 was compared with other populations. A frequency distribution of onset temperatures showed that nearly 62% of all the kernels analyzed (20 kernels per population × five populations = 100 kernels total) had a T_o of 60–65°C. Individual kernel data are not shown. Only a small number (about 3%) of the kernels analyzed showed a T_o of 59°C or less, and about 35% had a T_o of 66% or higher.

Krueger et al (1987a) reported an increase in T_o and ΔH and a decrease in range in dent corn lines that had undergone an annealing treatment. The shifts were attributed to an increase in crystallinity of the starch molecules that occurred because of the treatment. Marchant and Blanshard (1978) proposed that annealing permits partial melting of some crystallites and a general realignment of starch chains in the amorphous, or gel, phase, resulting in increased hydrogen bonding. The maize kernels in the current study had not been annealed, but all samples were treated similarly, so comparisons can be made among them. Perhaps some variations in the T_o, range, and ΔH among populations could result from differences in the native alignment and hydrogen bonding of the starch molecules.

In another study, gelatinization temperature and a ΔH of amylomaize were reported to increase as granular size decreased (Knutson et al 1982). They also suggested that gelatinization parameters are influenced by the impermeability of the starch granule surface.

Populations 83:3001, 83:3011, 83:3121, and 83:3031 were all grown in the same location during the same year, so growing conditions of the kernels were alike. The differences in DSC properties among these populations could be attributed to some structural variations of the starch. Krueger et al (1987a) also reported that starches from different maize varieties (before annealing) exhibited significant variations in thermal properties, especially in ΔH. Population 86-87:441 was grown in a different climate and year, so some of the large variation between it and the other populations could be due to environmental differences.

Starches from individual plants (representing five kernels) within a maize population exhibited significant variations in T_o, range, and ΔH except for 86-87:441. Genetic variability of the first four populations may account for these differences. All populations are genetically variable. Population 86-87:441 showed no significant differences in T_o, range, and ΔH among the plants that were analyzed, suggesting homogeneity of the genes controlling these properties.

TABLE III
Intrapopulation Variability in the Differential Scanning Calorimetry Properties of Maize Starch Plants

Plant[a]	T_o(°C)	$2(T_p - T_o)$ (°C)	ΔH (cal/g)
83:3001-5	59.8	14.6	1.83
83:3001-6	61.2	15.9	2.52
83:3001-7	59.6	17.2	2.39
83:3001-8	61.6	19.5	2.34
83:3001-9	62.8	12.7	2.64
83:3001-10	63.8	12.3	2.21
83:3001-11	63.8	14.0	2.63
83:3001-12	61.4	14.5	2.70
83:3001-13	63.2	15.4	2.57
83:3001-14	62.6	14.7	2.59
83:3001-15	64.5	13.2	2.56
83:3001-16	62.2	14.7	2.76
83:3001-17	64.4	12.4	2.64
83:3001-18	61.8	11.7	2.43
83:3001-19	61.0	14.0	2.48
83:3001-20	60.6	16.9	2.74
83:3001-21	62.5	12.7	2.64
83:3001-26	62.9	13.7	2.32
83:3001-35	63.0	14.4	2.83
83:3001-40	64.7	10.5	2.75
$LSD_{0.05}$	1.54	2.44	0.28

[a]Each plant represents mean data from five kernels.

Intrapopulation Variability of Maize

The variability within the same population was evaluated using 20 plants of 83:3001. Data are given in Table III. The T_o of 20 plants (representing the mean of five kernels each) ranged from 59.6 to 64.5°C. Significant differences were noticed in the T_o values as judged by LSD analysis at a significance level of $P < 0.05$. The gelatinization range was more variable than the other parameters, with a range of 11.7 to 19.5°C and an $LSD_{0.05}$ of 2.44°C. The values ranged from 1.83 to 2.83 cal/g, and a statistically significant variation in the ΔH ($LSD_{0.05} = 0.28$) was observed among plants of the same population. The frequency distribution of T_o of kernels (five kernels × 20 plants = 100 kernels) within the same population indicated that 88.3% of the kernels had a T_o between 60 and 65°C. Individual kernel data are not shown. Only 7.5% of the kernels had a T_o of 59°C or less, and 4.2% had a T_o of 66°C or higher. The gelatinization ranges varied considerably, but more than 50% of the kernels displayed a range of 13–16°C. About 75% of the kernels analyzed showed an energy of gelatinization of 2.3–2.8 cal/g. Most of the remaining kernels had a ΔH value between 1.8 and 2.3 cal/g.

The significant differences in T_o, range, and ΔH noted within this genetially variable population suggest that there may be many genes or gene interactions having a major effect on starch gelatinization behavior. As discussed earlier in this paper, genetic differences may account for variations in starch granule structure, size, molecular alignment, or hydrogen bonding, which, in turn, may influence gelatinization behavior. Krueger et al (1987a) isolated and analyzed raw starch from eight kernels of OH43. The T_o ranged from 61.7 to 65.9°C, with a mean of 63.3°C and a standard deviation of 1.31. The ΔH value ranged from 1.58 to 2.57 cal/g, with a mean value of 2.18 cal/g and a standard deviation of 0.319. Gelatinization properties varied significantly within the OH43 variety, which also is an inbred variety.

In conclusion, maize populations genetically variable for appearance and yield also were found to be variable in thermal properties, as measured by DSC. Significant differences ($P < 0.05$) in T_o, range, and ΔH were noted among the populations analyzed as well as anong plants within a population. The various responses to DSC analysis suggest that there are structural differences in the starch granules that may be controlled by genetic makeup. One population, 86-87:441, showed no statistical variation in thermal properties among plants, indicating homogeneity of genes or simply of those genes governing starch thermal behavior. Much more work is needed to study the genetic control of starch structure and the subsequent effect on thermal properties of starch.

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